Forensics and Homeland Security

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Program: Forensics and Homeland Security

Title: NIST Support of the CDC Laboratory Response Network for Chemical Terrorism

Authors: K.E. Murphy, M.M. Schantz, G.C. Turk, B.A. Benner, Jr., T.A. Butler, and L.J. Wood

Abstract: We have developed a new method for the determination of cyanide (CN) in human whole blood and used the methodology to evaluate the stability of CN in frozen blood-based proficiency standards. The proficiency standards are used by the Centers for Disease Control (CDC) to assess measurement capabilities of members of the Laboratory Response Network (LRN), a network of national laboratories that are equipped to rapidly test for human exposure to chemical and biological weapons. CN has a long history of use as a chemical weapon and the need for methods and standards for measurement of CN was identified by CDC as a top priority. CN exposure is indicated by measuring the CN content of whole blood, however CN is very reactive and blood CN concentrations can decrease or increase over time depending on the method of storage. As a result, there are no commercially available CN standards to underpin the accuracy of blood CN measurements. CDC contracted a commercial standards supplier to produce a suite of blood-based CN standards. The standards are composed of a blank level and three elevated levels containing nominally 0.075 mg/kg CN, 0.3 mg/kg CN, and 1.5 mg/kg CN. Standards are supplied as 0.5-mL aliquots of the CN-spiked blood packaged in 5-mL head space vials and stored frozen at –50 °C. We have accurately measured the CN content of each level and have monitored the CN concentrations for over one year.

Purpose: CN is a likely chemical terrorism agent due to its toxicity, wide availability, and history of use as a chemical weapon. Fatalities from exposure to CN may be prevented by the administration of antidotes if exposure is diagnosed in a timely manner. Rapid response requires a well coordinated public health system. The anthrax incident in 2001 showed that laboratory capacities can be quickly overwhelmed if such coordination is lacking. Consequently there is a need for an organized network of laboratories with the capabilities to rapidly and accurately measure terrorism agents such as CN. New methods and standards are required to support and critically evaluate public health laboratories measurement capabilities.

Major Accomplishments: We have developed a new method for the measurement of CN based on headspace gas chromatography/mass spectrometry (GC/MS) using a labeled cyanide internal standard ($K^{13}C^{15}N$). The method has been adapted by CDC for use by laboratories in the LRN. In addition we have completed measurement of the CN content and stability of CDC supplied proficiency standards. Measurements of five samples from each level were performed on a bi-monthly basis for a period of 14 months. Results showing the stability of CN in the proficiency standards are depicted in Figure 1. The measured CN concentration is plotted versus analysis date. The solid black line shows the trend in CN concentration during the 14-month period for the blank level as well as the three elevated levels. The CN concentration remained stable for the nominal 0.3 mg/kg and 1.5 mg/kg levels, but increased slightly for the blank and nominal 0.075 mg/kg level. The average CN concentration for all levels was higher than the target nominal values. This may be partly due to endogenous CN in the blood stock, but may also be a result of the artifactual production of CN from freezing the blood. Overall, results for the three elevated

levels show a variability of less than 6 % relative (1s, n = 33). These results demonstrate a considerable improvement over data reported in the literature for the stability of CN-spiked blood standards and validate the viability of storage at –50 °C.

Impact: An integrated public health system is vital in the event of a chemical terrorism attack. In the past two years Congress has appropriated $95 million for chemical terrorism preparedness. There are currently 62 public health laboratories in the chemical component of the LRN, over 40 of which are Level 2 designate laboratories. Level 2 laboratories are tasked with the analysis of CN, toxic metals and lewisite in human samples. Inclusion in the network requires participation in a rigorous quality assurance program, which includes the analysis of proficiency standards. The data NIST has provided to characterize the CDC blood-based proficiency standards will help ensure that network laboratories provide accurate measurements in the event of a chemical terrorism incident involving CN.

Future Plans: NIST will continue to monitor and respond to future measurement and standards needs of the CDC Laboratory Response Network.

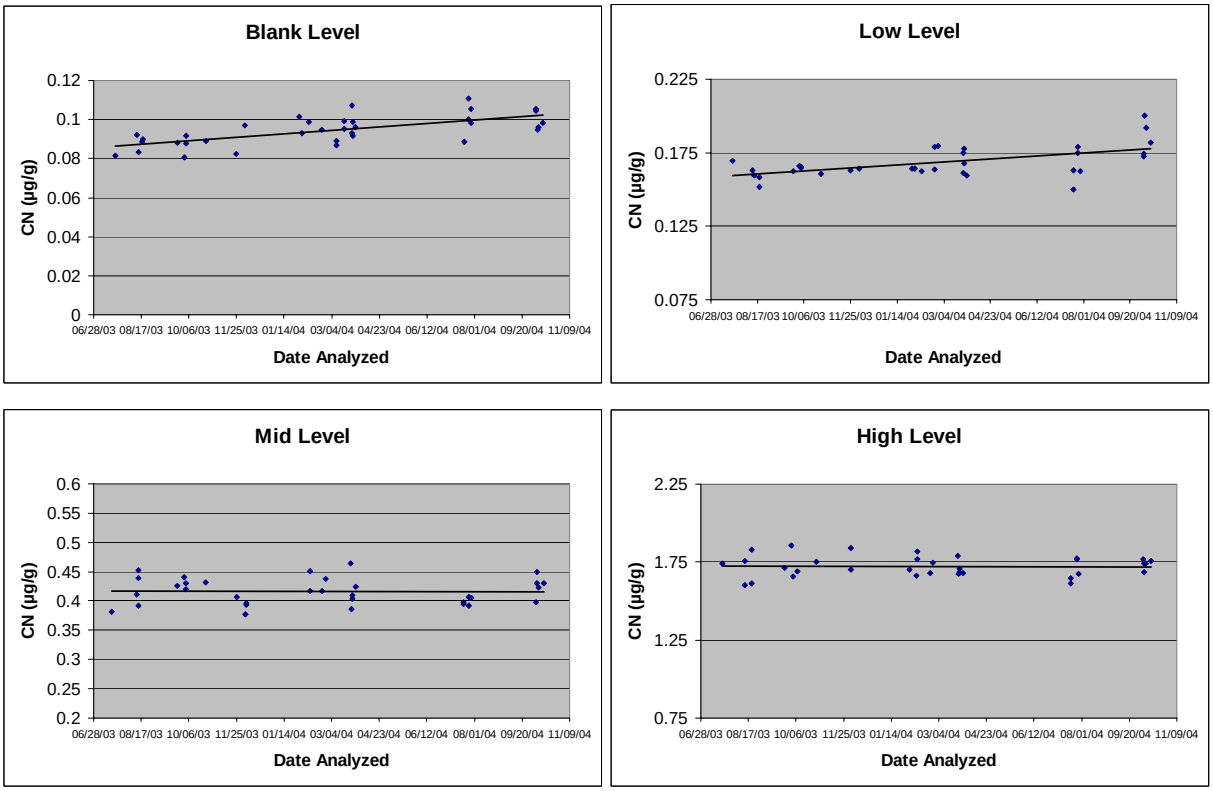


Figure 1. Plots showing the stability of CN in frozen blood-based proficiency standards over a 14-month period.

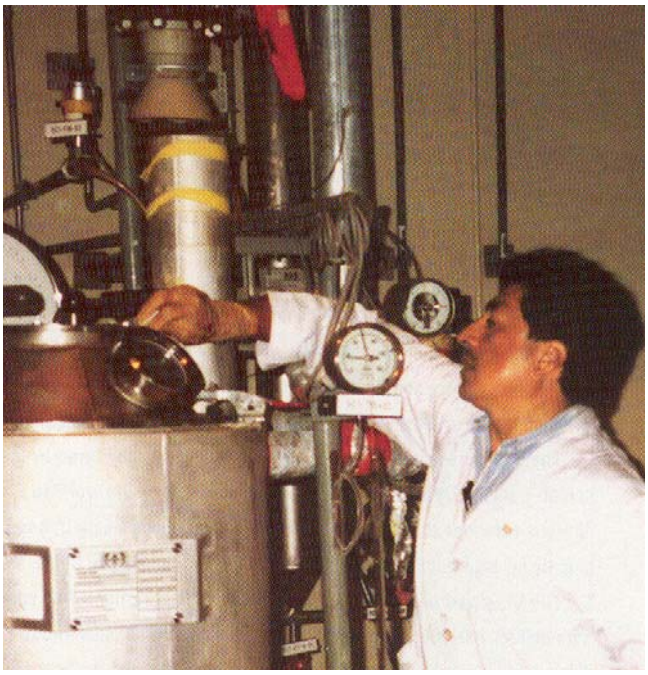
Program: Forensics and Homeland Security

Title: Determination of Traces of Fissionable Materials Using Delayed Neutron Activation Analysis

Authors: R.M. Lindstrom, E.A. Mackey, and G.P. Lamaze

Abstract: Detection and measurement of small traces of fissionable uranium and plutonium can be done by delayed neutron activation analysis. The method is intrinsically specific to nuclear fission, the sensitivity is excellent, and the procedure is simple, rapid, and readily automated for high throughput.

Purpose: As shown in the figure, an important tool in nuclear forensics is the collection and analysis of "swipe" samples at sites where materials of interest may be, or may have been at one time. Tiny traces of fissionable uranium or plutonium can be left behind whenever these materials are handled or transported. Using neutrons from the NIST research reactor, the delayed neutrons from fission in these traces can be used to detect and quantitate U and Pu in swipe samples with excellent speed, sensitivity, and specificity.



IAEA inspector collecting a swipe sample from process equipment in a nuclear fuel fabrication facility in 1994 (*Anal. Chem.* **74** (2002), 28A)

Major accomplishments:

Delayed Neutron Activation Analysis is being established at NIST for the measurement of small quantities of fissionable nuclides such as ^{235}U and ^{239}Pu. After a brief neutron irradiation, the sample is placed quickly into a neutron detector array and the neutron emission rate measured and compared with that of a standard. The method is well-tested, rapid, specific, matrix independent, nondestructive, and sensitive. The system being built at NIST is calculated to have a detection limit for either of these species about 10 picograms, based on a straightforward extrapolation from published practice. The analysis time is less than 2 minutes per sample.

Preliminary tests have been performed using uranium standards prepared by depositing solutions containing known amounts of uranium onto filter papers, irradiating, and counting on a detection system with one ^{3}He detector surrounded by hydrogenous moderator. Two shielding configurations were tested, one using water as the moderator and the other using polyethylene. Based on these results and a review of the literature, a final detection system was designed. This consists of ten neutron detectors in a 30 x 30 cm cylindrical moderator of polyethylene, lined with 2 cm of lead to absorb gamma radiation. The design of the manual system incorporates the existing pneumatic rabbit assembly for irradiation control. Incorporated in its design is the ability

to unload a sample from the receiver after irradiation with compressed air. This feature will be used to move the sample rapidly to the neutron detector through a polyethylene flight tube.

Impact: On completion and verification, the Nation will have a readily accessible, rapid means of measuring traces of fissionable U and Pu in samples of forensic interest. In addition, the specificity and sensitivity of this method of analysis will be put to use in certifying trace uranium in Standard Reference Materials.

Future plans: To improve sample throughput, the transfer system can be readily automated with computer control in the coming year. It has been demonstrated elsewhere that ^{233}U, ^{235}U, and ^{239}Pu can be distinguished by the relative yields of delayed neutron precursors with different half-lives, and also of several fission products. We plan to add a gamma-ray detector into the neutron moderator to exploit this signature.

Program: Forensics and Homeland Security

Title: A NIST Reference Material to Support Explosive Device Measurements

Authors: W.A. MacCrehan and M. Bedner

Vision: To assure the best measurements to detect and investigate explosives crime.

Purpose: To deter explosives crime, national, state and local forensic laboratories measure the additives in smokeless powder (gunpowder). US military laboratories also measure the chemical composition of such propellant powders to determine the stability of munitions. CSTL is providing a reference material, RM 8107 Additives in Smokeless Powder to support the quality of these forensic and military smokeless powder measurements.

Pipe Bomb Ingredients and RM 8107

Major Accomplishments: This new reference material consists of 5 g of a rifle-type smokeless powder. Reference values were assigned for four commonly determined additives, nitroglycerin, diphenylamine, N-nitrosodiphenylamine, and ethyl centralite. An ultrasonic solvent extraction method was developed to quantitatively recover these analytes. Mean values determined by micellar capillary electrophoresis and liquid chromatography were in good agreement. Since the LC technique provided lower uncertainty in the measurements, it was used for the final value assignment. RM 8107 was released for sale August 20, 2004.

Impact: With the development of this smokeless powder RM, forensic laboratories will have a reliable material for the development and validation of measurement methods for improvised explosives devices. The RM may also be used as a proficiency challenge sample to test operator and laboratory performance in explosives measurements as part of laboratory accreditation activities under ASCLD/LAB. In addition, US military laboratories can use the additive reference values in the NIST RM to assure accurate measurement to assure the stability of munitions.

Future Plans: The smokeless powder RM 8107 was developed to help assure the quality of 'low explosives' type measurements. However, terrorist explosives' incidents often involve the use of 'high explosives' such as TNT and RDX (ingredients of military and plastic explosives). In conjunction with OLES and the Department of Homeland Security, we are developing a reference material for these high explosives. An inert solid matrix will be coated with commonly used military explosives. The goal is to develop a particulate explosives material that can be used to test explosives detection equipment used for airport security screening, evaluation of suspicious packages by first responders, and forensic laboratory post-blast investigations.

Program: Forensics and Homeland Security

Title: Raman Libraries

Author: S.J. Choquette

Vision: To develop instrument independent Raman spectral libraries and the calibration protocols to enable their use with field portable instrumentation.

Purpose: Portable Raman spectrometers are currently in use by first responders—hazardous materials (hazmat) specialists, law enforcement officers, safety and security personnel—to identify unknown substances in real-time with minimal handling. Such materials include explosives, drugs of abuse, and bio/chemical agents. Our goal is to provide both the physical standards and the validated Raman spectral libraries necessary to impart confidence in Raman measurements, to provide measurement traceability to national standards, to improve the Raman determination efficacy, and to ensure evidentiary acceptance of Raman measurements.

Background: The issue with spectral libraries in general, is that the origin, purity, and physical state of the compound are typically never specified. It is also quite infrequent that the instrument state of the analyzing spectrometer is adequately known. Although parameters such as spectral resolution may be listed, the calibration state of either axis (wavelength and intensity) are rarely given. Quality systems, which might assure the veracity of the data, are infrequently, if ever, used for spectral acquisition. Raman spectral libraries have the additional challenge in that the spectra are instrument dependent, due to the variety of laser excitation wavelengths used and nature of the measurement. Because Raman is an emission process, the spectra are convolved with the instrument response. This is quite varied from vendor to vendor, even given the same laser excitation wavelength. Our goal is to develop a standardized protocol for measurement of these compounds by Raman spectroscopy and then develop the protocols for transferring this reference data to commercial instrumentation.

Major Accomplishments 2004: In 2004 a test set of compounds comprising 14 persistent organic pollutants (pop's) was chosen for assessment and to aid in the development of standardized protocols for inclusion of compounds into a spectral library. Our next goals are to study efficient methods for transferring this data to the commercial sector. These compounds were chosen based upon input from Raman vendors and NIST's prior experience in developing SRM's based upon these materials. GC-FID and differential scanning calorimetry were employed to verify the purity of the materials. Raman spectra were acquired on research grade instruments employing 514 nm, 785 nm and 1064 nm laser irradiation. Each instrument was calibrated in both x and y axes using NIST SRM's. Spectra were corrected for scattering and incorporated into a searchable library. Arrangements are in progress to measure these compounds upon commercial grade systems to assess the efficacy of software algorithms to identify these compounds utilizing this reference data.

Future Plans. A collaboration initiated with applied math (Anthony Kearsley) to develop algorithms for automated Raman spectral searches. This is an important and necessary extension of this work to compare instruments of widely disparate capabilities.

Program: Forensics and Homeland Security

Title: Microfluidic Devices for Rapid DNA Analysis for Human Identification

Authors: W.N. Vreeland and L.E. Locascio (839); M.Gaitan, J. Geist, and J. Shah (812); N. Y. Morgan, P. Smith, T. Pohida, and J. Kakareka (NIH); and C.W. Kan, A. E. Barron (Northwestern University)

Abstract: We have developed a new microfluidic device with auxiliary optics, pneumatics, and software to allow for rapid analysis of DNA "fingerprints" for human identification. The microfluidic devices are fabricated from common low-cost commercial plastics facilitating their application as single-use devices and thereby eliminating concerns of sample-to-sample cross contamination. Current device design and configuration are approaching the performance in salient figures-of-merit to current state-of-the-art equipment with the time required for analysis being reduced by nearly 90%.

Purpose: The National Institute of Justice estimates a backlog of a 542,700 cases for DNA analysis as of April 2004 and current forensic crime labs do not have the equipment capacity to address this backlog in a timely manner. Forensic DNA analysis or "fingerprinting" involves the measurement of the molecular size of several fragments of DNA produced in a specially designed molecular-biological reaction. NIST's development of a microfluidic device for forensic DNA analysis is addressing this backlog by allowing for analysis techniques that are both faster and more economical, while still ensuring the data created is of the highest quality.

Major Accomplishments: The plastics that are used to fabricate NIST's microfluidic devices must fulfill a variety of chemical, mechanical, and optical properties. In particular they must have (1) low permeability to aqueous buffers and salts, (2) low electrical conductivity, (3) glass-transition temperature between 110 and 120 degrees Celsius, (4) low optical fluorescence, (5) high optical transparency, and (6) uniform thicknesses. Several commercial plastics were screened, and a medical-grade poly(methlymethacrylate) was determined to be the best match to these criteria (this is the same plastic that is used to fabricate disposable contact lenses for vision correction).

The microfluidic channel must also be of a particular architecture and design (see Figure 1 for a general device layout). Two physical parameters of the device design are important, including separation channel length and sample injector size. The separation channel length determines the time required for the analysis as well as its analytical selectivity, while the sample injector determines the sensitivity and efficiency. In both cases, the desirable aspect of one parameter is increased at the cost of the other. We have design, fabricated and tested a systematic assortment of microfluidic channel designs to determine the most appropriate physical parameters for the NIST forensic DNA analysis. The final device design had an injector size of 100 microns and a separation channel length of ~ 9 cm. Results from this device are presented in Figure 2.

Further we have designed, assembled and tested the necessary optical detection system for fluorescence detection of the DNA "fingerprinting" fragments. The current system consists of a ball lens that collects the microchannel fluorescence and guides that light down an optical fiber

that is then imaged on a CCD camera with an optical grating. This system allows for detection of an arbitrary number of channels simultaneously. This approach allows for large scalability in future device designs and has no moving parts (in contrast to all other optical detection strategies in current commercial use). The result is a more rugged device as is required for the ultimate deployment of this device in the field.

Figures

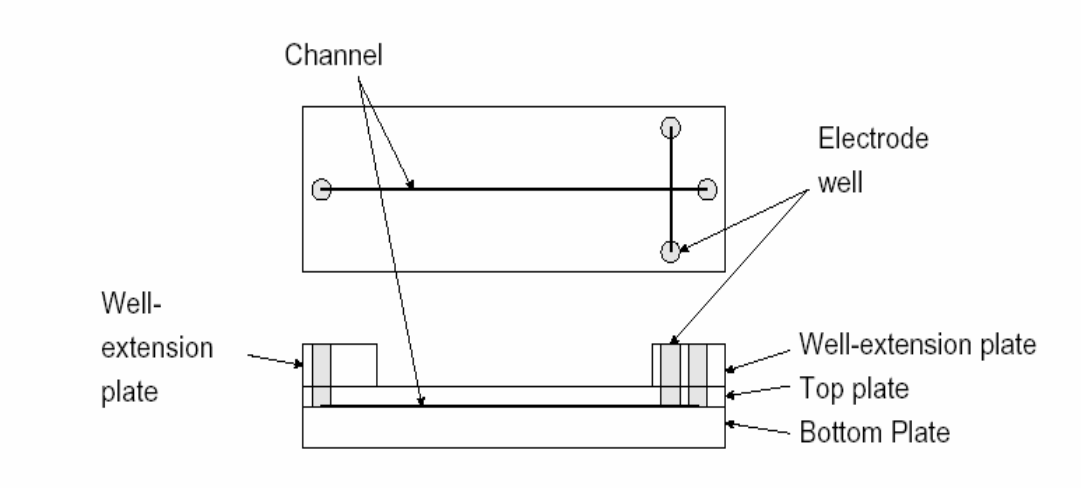


Figure 1. Top and side view of the single-channel microfluidic device currently being used in DNA separation experiments at NIST (not to scale). All lines are shown because the plastic substrates from which the device is made are transparent. The electrode wells, denoted as shaded circles, allow for the application of appropriate electrical fields. The DNA injector is formed from the intersection of horizontal and vertical channel (denoted as black lines) and the right-most portion of the horizontal line represents the separation channel

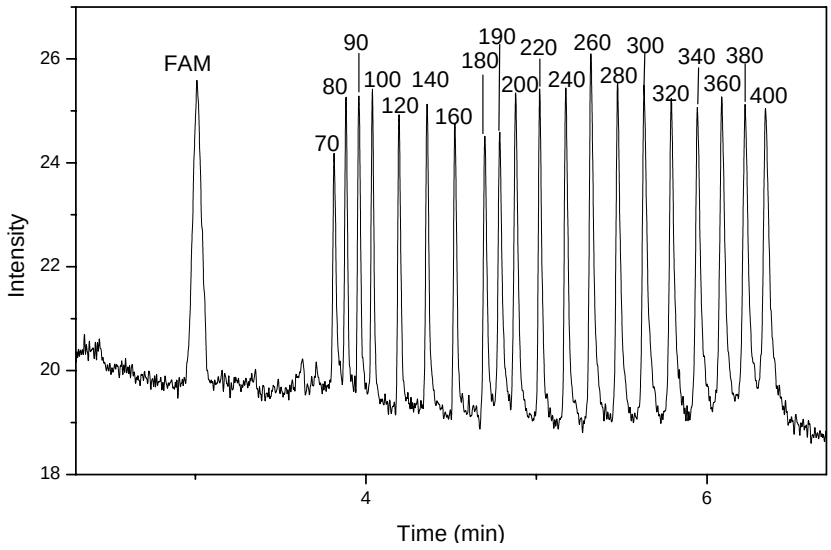


Figure 2. A demonstration of the NIST microfluidic DNA analyzer, showing the separation of a DNA "ladder" consisting of a variety of fragments of increasing size. Each peak represents a DNA fragment of particular size with larger fragments appearing later in time. The total analysis is performed in approximately 7 minutes.

Program: Forensics and Homeland Security

Title: Ethanol in Water

Author: M.M. Schantz

Abstract: Accurate calibration of instrumentation is critical in areas of forensic testing where quantitative analysis directly affects criminal prosecutions, as is the case with the determination of ethanol in blood and breath. Blood- and breath-alcohol testing can be imposed on individuals operating private vehicles such as cars, boats, or snowmobiles, or operators of commercial vehicles like trucks, planes, and ships. Two new ethanol in water SRMs, SRM 1828b and SRM 1847, with six and three concentration levels, respectively, have been issued to replace the previous SRM 1828a, which had only four concentrations levels.

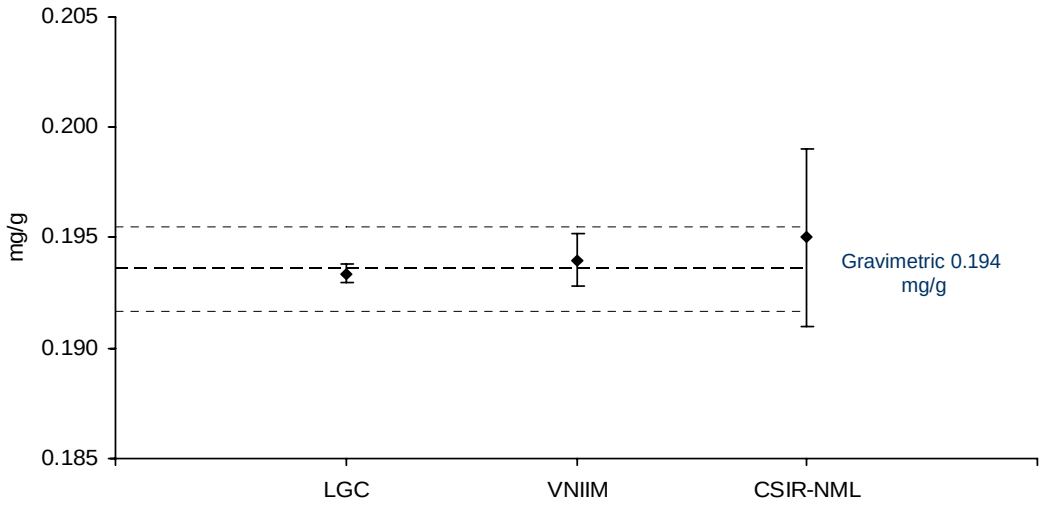
Purpose: The various levels of blood-alcohol that determine whether these operators are considered legally impaired vary depending on the circumstances, state, and even month in which the testing is occurring. As a result, practitioners in the field of alcohol testing have a need for reliable and stable standards at several concentrations. Most blood alcohol levels in driving under the influence (DUI) cases fall in the range of 0.1% to 0.3% (the average blood alcohol for a DUI traffic stop is 0.16 % to 0.17%). By providing SRMs with concentration levels set to legally relevant points, the accuracy of blood- and breath-alcohol testing will be improved.

Major Accomplishments: The concentration levels in SRM 1828b, Ethanol-Water Solutions (Blood-Alcohol Testing: Six Levels), have been tailored to legally relevant points, specifically 0.02% and 0.04% for "zero tolerance" and occupational alcohol testing, 0.08% and 0.1% for state drunk driving laws, and 0.2% and 0.3% for an average and high level for blood alcohol measurements. In addition, three concentration levels of ethanol in water (2%, 6%, and 25%) have been prepared as SRM 1847, Ethanol-Water Solutions (Breath-Alcohol Testing: Three Levels), for use as reference solutions for breath-alcohol instruments. The SRMs were prepared gravimetrically, and the concentrations of ethanol in water were confirmed at NIST by using gas chromatography with flame ionization detection, the analytical method of choice for blood- and breath-alcohol testing in the forensic laboratory. Because some laboratories, particularly in California, are required to verify their primary standards of ethanol in water by using titrimetry, the National Metrology Laboratory (CSIR-NML) in Pretoria, South Africa provided measurements for each of the nine concentrations of ethanol in water using a titrimetric method, shown to be very precise and accurate. The National Analytical Reference Laboratory (NARL) in Sydney, Australia (another national metrology laboratory) also provided measurements for four of the solutions (0.08%, 0.1%, 0.2%, and 6%) using an exact matching isotope dilution-gas chromatographic method, also demonstrated to be a very precise and accurate method. The certified concentrations of ethanol in water the SRMs are based on a combination of the gravimetry, NIST, CSIR-NML, and NARL measurements. The relative expanded uncertainties for the certified concentrations are less than 1.2% for each concentration level.

Impact: The redesigned concentration levels in SRM 1828b and SRM 1847 have made these SRMs more useful to the forensic community for use as reference solutions for breath-alcohol instruments.

Future Plans: An additional solution of the ethanol in water azeotrope will be made available in FY05 as SRM 2900. Feedback from customers indicated that the azeotropic mixture is needed in addition to those levels now available in SRMs 1828b and SRM 1847 for use as a starting point in the preparation of the customers' working calibration solutions.

Results for CCQM-K27-Subsequent study level 1 showing gravimetric value and upper and lower limits of the expanded uncertainty of the gravimetric value based on the CCQM-K27a study



Program: Forensics and Homeland Security

Title: Candidate Material for an Explosive Residues on Soil Reference Material.

Authors: B.A. Benner, Jr. and W.A. MacCrehan

Abstract: A bulk soil sample was collected, processed, and characterized for a number of explosives and their degradation products. This material will be used as a candidate reference material to support the measurement of explosives in soil. Preliminary measurements to characterize this material have been completed.

Purpose: To provide a candidate material for development of a NIST Reference Material (RM) for environmental and forensic explosives analysis. This RM will support measurements required by the demilitarization of former munitions proving grounds and trace explosives detection.

Major Accomplishments: A total of approximately 45 kg of bulk soil was collected at a military munitions proving ground. The bulk material was dried and a portion was sieved to particle sizes between 90 µm and 212 µm, mixed, and irradiated by a standard ^{60}Co procedure to minimize microbial activity that might change the levels of analytes upon storage. This processing yielded about 1.5 kg of suitable candidate material. The concentrations of key explosive compounds were evaluated in this candidate material. One-gram sub-samples were solvent extracted and analyzed by gas chromatography with negative ion chemical ionization-mass spectrometric (GC/NICI-MS) detection for measurement of explosive compounds, including 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX), and related degradation products. The concentrations of the nine analytes including the high explosives, smokeless powder additives, and their microbial degradation products were determined to be in the range of ng/g to µg/g.

Impact: Recently, concerns have been raised by the detection of explosive compounds in the ground water surrounding our military munitions facilities (1). There is insufficient understanding of the environmental fate of the components leaching of incompletely and unexploded ordinance. An explosives on soil material would support the explosive residues measurements in the on-going remediation of these munition proving grounds (2). In addition, this material would be appropriate for use in verifying measurements that are part of landmine detection, forensic post-blast investigations, and trace explosives residue detection.

Future Plans: We will continue to refine our GC/NICI-MS and liquid chromatography/mass spectrometric (LC/MS) methods for measurements of these explosive analytes in this natural matrix material. Evaluation of the long-term stability of this soil will be an important component of the development of a reference material for these unstable analytes. It will be interesting to see if the ^{60}Co irradiation was successful in eliminating any further microbial degradation of 2,4,6-trinitrotoluene (TNT) that was noted in this soil prior to irradiation.

References:

1. MacDonald, J. A. “Cleaning Up Unexploded Ordnance,” Environ. Sci. Technol., 35, 370A-374A (2001).

2. Environmental Protection Agency (EPA) Methods 8095 (Explosives by Gas Chromatography) and 8330 (Nitroaromatics and Nitramines by HPLC).

Program: Forensics and Homeland Security

Title: Evaluation of Oleoresin Capsicum (Pepper Spray) Canisters for Chemical Content And Reliability

Authors: E. White V, R.J. Christensen (retired), K.W. Phinney, B.J. Porter, C. Presser (836), L.C. Sander, and R.Q. Thompson (guest researcher)

Abstract: A program to characterize the chemical compositions and the physical characteristics of pepper spray products has been established in the Analytical Chemistry Division at the request of the NIST Office of Law Enforcement Standards and with funding from the National Institute of Justice. A variety of products representing a cross section of those used by law enforcement agencies have been selected for study. The identities and concentrations of the active ingredients are determined by liquid chromatography/electrospray mass spectrometry and the identities of carriers and propellants by gas chromatography. Physical testing includes measurement of the number of 1-second bursts in a canister, a range test, a spray pattern test, drop tests, and, for the products that produce fogs, the droplet size. Improved analytical methods have been developed for the determination of the active ingredients.

Purpose: Pepper Spray is used by law enforcement officers to subdue non-cooperative individuals. It is effective 85-90% of the time and its use reduces the number of injuries to officers and suspects, and the number use-of-force complaints. The causes of failures have not been established, but may include low concentrations of the active ingredients, poor delivery, and variable subject response. The goal of this study is the determination of the identities and concentrations of the ingredients and the mechanical performance of pepper spray units to establish potential modes of failure and hazards in handling.

Accomplishments: An improved liquid chromatographic (LC) separation for eight naturally occurring capsaicinoids, the active ingredients in pepper spray, and two internal standards, N-vanillyl octanamide and decanamide, has been developed. Satisfactory quantitative results have been demonstrated for the measurement of the analytes in mixtures with positive ion atomospheric pressure ionization electrospray mass spectrometry (API-ES-MS) for detection. Authentic samples of the compounds have been purchased or synthesized and their purity determined. The analytical method has been tested on several naturally occurring capsaicin mixtures and on pepper sprays.

New instrumentation has been developed to support this effort. A test chamber has been constructed for physical testing. Semi-automated devices have been fabricated and tested for determining canister spray capacity, for performing pattern and range tests, and for performing drop tests. Environmental chambers have been constructed for storage of canisters under controlled conditions. Phase Doppler interferometry instrumentation is presently being set up to measure particle sizes near the point of impact within the test chamber.

Tests have been performed on several canisters that have failed in actual field use. The composition of one such canister, reported to have caused injuries during a training exercise, was characterized by API-ES-MS. The spray was consistent with a synthetic (rather than natural)

product, and the total level of active constituents was comparable to sprays formulated with natural pepper extracts. A second canister reported to have failed during use was found to exhibit a weak spray pattern that was not centered in the direction the canister was aimed.

Impact: A reliable set of quantitative chemical and physical measurements on a representative set of canisters is expected to provide a basis for determining the most likely causes of failures in the field and to provide a benchmark against which manufacturers can assess the performance of their products.

Future Plans: Chemical and physical measurements are scheduled for approximately 1000 canisters. The resulting data is expected to provide indications of the presence/absence of design flaws or manufacturing defects that might lead to failures. The facility and capabilities will be made available to assist in determining the likely cause of future reported failures.